BIOMEDICAL RESEARCH IN THE SEA, A SEARCH FOR DRUGS AND NOVEL COMPOUNDS

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Biomedical research has discovered numerous pharmaceutical drugs and novel compounds produced by living organisms. Most of this research has focused on terrestrial sources but a potential reservoir of untapped drugs is being discovered in the oceans. Compounds produced from marine sponges, tunicates, bryozoans, soft corals and algae are being tested as potential agents against cancer, AIDS and other diseases. In this effort, the goal of the Division of Biomedical Marine Research (DBMR) at Harbor Branch Oceanographic Institution (HBOI) is to isolate and identify bioactive compounds from marine organisms. Since 1984 over 16,000 macroorganisms have been collected worldwide by HBOI personnel using scuba, JOHNSON-SEA-LINK submersibles and ROVs to depths of 915m. Biological assays of samples collected determine potential leads for antitumor, antiviral, antifungal, antibacterial and immunomodulatory agents. Active compounds are then isolated and structurally identified by nuclear magnetic resonance and mass spectroscopies. Presently over 200 chemical structures of biologically active compounds have been determined by DBMR scientists and affiliates resulting in 14 issued and allowed patents and 44 publications.

INTRODUCTION

Plant and animal sources from the land and the sea hold promise for novel compounds that may be developed as pharmaceutical drugs. Cancer, heart disease, and viruses such as AIDS are among the leading causes of death in the western world; new drugs and therapies are necessary to make progress on these disease areas (Fisher, 1988; Suffness and Thompson, 1988).

Historically, natural products have been used in Chinese folklore medicine for over a thousand years (Kim, 1967). By the 1800's, scientific interest developed in the secondary metabolites of terrestrial plants and animals as a source of novel and biologically active compounds (Fenical, 1982). It has only been since the 1950's, however, that natural product research has looked to the sea. Marine natural product research rapidly accelerated with the motivation of discovering an abundance of novel chemical structures with potential biological activity (Bergquist and Wells, 1983). The advent of new technologies for underwater research, *e.g.*, scuba and submersibles, further enhanced the capabilities of this new field.

Bergmann led this research with the isolation of the nucleoside spongothymidine from the marine sponge *Cryptotethya crypta* (Bergmann and Feeney, 1950). This nucleoside later served as a model for the synthesis of two drugs with antiviral and

antitumor activities (Rinehart and Shield, 1987). Random surveys for marine organisms with antimicrobial, antifungal, cytotoxic, and antitumor activities ensued. The first systematic investigations of biologically active marine organisms began in the 1970's. The R/V ALPHA HELIX Baja expedition in 1974 assayed 850 marine species for antimicrobial and antifungal activities, and in 1978 an antiviral investigation of Caribbean marine organisms was initiated (Rinehart and Shield, 1987). An important aspect of these expeditions was that the samples were screened on board to prevent possible degradation of active compounds.

Since the 1950's, thousands of novel compounds have been discovered from marine natural products. These have been summarized in numerous reviews for various taxa (Faulkner, 1984a- algae; Faulkner, 1984b- invertebrates). Also included are reviews of novel marine compounds with various bioactive properties (Moore, 1981; Shimizu and Kamiya, 1983; Munro *et al.*, 1987; Paul and Fenical, 1987).

A resurgence in interest in marine-derived pharmaceuticals has developed in the last decade. One result is that many compounds that were tested previously have been retested for new activities. In addition, new screens have been developed to better define a compound's mechanism of action against a particular disease. In this effort the National Cancer Institute (NCI) is continuing to discover and develop anticancer and anti-AIDS agents. NCI's empirical drug discovery program has collected and tested nearly 18,000 marine organisms for antitumor activity using the *in vitro* P388 mouse leukemia virus (Suffness and Thompson, 1988). NCI is now adding to their biological assays a panel of 100 human tumor cell lines. Also, recent emphasis has been placed on extensive collections over a wide range of depths and geographical localities.

Although a recent newcomer to this field, Harbor Branch Oceanographic Institution (HBOI) initiated in 1984 a program dedicated to the discovery of new drugs from marine organisms. The Division of Biomedical Marine Research (DBMR) at HBOI uses a variety of screens to test marine derived compounds in four disease areas: antitumor, antiviral, antifungal/bacterial, and immunomodulatory. Programs also include fermentation of marine bacteria, fungi and blue green algae, sponge cell culture, molecular biology, chemical ecology, and chemotaxonomy.

A collection program at HBOI utilizing the JOHNSON-SEA-LINK (JSL) submersibles and scuba has enabled HBOI/DBMR scientists to sample over 16,000 marine organisms from around the world to depths of 915m. HBOI has supplied NCI's anticancer program with deep and shallow marine organisms as well. DBMR's staff of 26 biologists, chemists, and microbiologists collect, screen, purify, and structurally identify the novel biologically active compounds from these samples. This paper summarizes some of the results from the first five years of HBOI's research on marine natural products.

METHODS

Collections

Samples were collected with the JOHNSON-SEA-LINK (JSL) submersibles, HYSUB ROV, scuba, snorkel and wading, dredge and trawl. The 4-person JSL submersible, owned and operated by HBOI, is capable of diving to 915m and is armed with an array of photographic and collection equipment including manipulator arm with clam-shell grab and suction hose; 12-rotating collection buckets; environmental data recorder to log temperature, conductivity, salinity, depth and light; and 35mm and color video camera systems (Tietze and Clark, 1986). HBOI's HYSUB 40 ROV is a tethered

vehicle with similar depth, photographic and collection capabilities as the JSLs (Clark and Schilling, 1987).

Scuba was a major collection method at depths less than 45m utilizing DBMR's chemists and biologists as divers. HBOI, an AAUS member organization, previously utilized the British Air Decompression Tables but in June 1988 adopted the DCIEM (Canadian) Tables. These tables are more conservative than the USN Tables and allow for multi-level diving. Dredges and otter trawls were used only on a few occasions when scuba or submersibles were not feasible.

Sample Processing

Details of sample collection techniques, processing, and documentation were developed by HBOI and used in NCI's contract for deep- and shallow-water collections of marine organisms for anticancer research (Pomponi, 1988). It is imperative that samples for natural product drug research are well documented and properly stored.

In situ documentation for HBOI collections generally consists of 35mm slides; videotapes; environmental data logs; descriptions of organism morphology, color, abundance, ecology and symbiotic relationships. Each sample was labeled with a collection number and photographed on deck. Taxonomic vouchers for identification and museum specimens were relaxed, if necessary, and preserved in formalin or ethanol. All samples, sites, and bioactivity data were recorded onto a computerized database for ease of data manipulation. Samples and extracts were stored at -20°C.

Bioassays

Samples were pulverized with a Virtis grinder and were extracted with various solvents (*e.g.*, ethanol, methanol, toluene). On most expeditions on HBOI vessels (R/Vs SEWARD JOHNSON, EDWIN LINK, SEA DIVER) samples were assayed on board immediately after collection. In 1988 samples were screened against the following assays to test for biologically active compounds:

Antitumor: P388, mouse leukemia cells

Antiviral:

1) HSV-1, *Herpes simplex* type 1 (DNA type)
2) A59, mouse hepatitus virus (RNA type)
3) FELV, feline leukemia virus (RNA type)
4) PR8, influenza virus type A (RNA type)
5) ADNO, human adenovirus (DNA type)

Antibacterial:

1) EC, *Escherichia coli* (gram - bacterium), disc method
2) BS, *Bacillus subtilus* (gram + bacterium), disc method
3) PA, *Pseudomonas aeroginosa* (gram - bacterium), disc method

Antifungal:

1) AN, *Aspergillus nidulans* (filamentous fungus), disc method
2) CA, *Candida albicans* (pathogenic fungus), liquid broth method
3) RPMI, Specific culture media for CA assay, liquid broth method

Immunomodulatory-

1) MLR, mixed lymphocyte response to determine:
   - ID - immunosuppression
   - IS - immunostimulation
   - ID - IS - both responses
   - IS/NS - nonspecific-IS
2) LCV, lymphocyte viability (cytotoxicity)
3) Mitogen panel.

These assays are used as primary screens because they are relatively rapid, reproducible, and reliable.

Chemical Isolation and Elucidation

Samples that were active from the primary screens were prioritized for further chemical isolation and finally structure elucidation by DBMR chemists. Bioactive compounds were isolated from the crude extract through purification steps using extractions and various chromatography techniques. Once isolated, the chemical structure was determined. IR, UV and mass spectroscopies were used to define functional groups and formulae. Nuclear magnetic resonance spectroscopy (NMR) was used to determine total structure. If the compound was crystalline, then the structure may have been elucidated by x-ray crystallography.

Microorganism Culture

Micro-algae, fungi, bacteria, and actinomycetes were isolated from sediments, macroorganisms and other environmental samples. Generally, the samples were ground or sonicated, aliquots plated on various agarized media, and incubated. Individual colonies or strains were purified by restreaking on various media under various conditions, *e.g.*, antibiotics, temperature, and light. Selected strains were then scaled up, *i.e.*, volume increased, to use in the assay program.

Sponge Culture

DBMR's sponge cell culture program was initiated in 1988. Various techniques for cell dissolution, microbial contaminant control, and media growth are being used to determine the feasibility of culturing bioactive sponges.

RESULTS

Collection Summary

Between March 1984 and July 1989 HBOI/DBMR personnel completed 30 expeditions for collections of marine organisms to discover novel bioactive compounds. Geographical locations included the Bahama Islands, Belize, Grand Cayman Island, Venezuela, Colombia, Lesser Antilles, Gulf of Mexico, Florida, South Carolina, Georgia, Alaska, Spain, Galapagos Islands, Cocos Island (Costa Rica), Pearl Islands (Panama), American Samoa, Australia, New Zealand, and Seychelles. A wide diversity of marine and brackish habitats were sampled including deep continental and island slopes, deep and shallow fore-reef slopes and escarpments, fringing and platform reefs, patch reefs, deep-water *Oculina* and *Lophelia* coral banks, rhodolith banks, caves, grassbeds, oyster beds, kelp forests, mangrove lagoons, sand and mud flats, hardgrounds, intertidal rocks, wrecks, pilings, buoys, and jetties.

During this five year period 16,670 marine macroinvertebrates and macroalgae were collected. Not included in these results are 1800 shallow-water samples collected in Australia and New Zealand through a subcontract with HBOI for NCI's anticancer collection contract. A total of 1591 scuba dives for a bottom time of 955.9 hours was compiled along with 243 JSL submersible dives (Table 1). The JSL collections to depths of 915m accounted for 4613 samples (27.7% of total) while scuba and snorkeling collected 10,882 samples (47.7% and 17.6%, respectively). Trawl and dredge were only used on four expeditions collecting 979 samples from 5 to 168m. The HBOI HYSUB ROV was used extensively only on one cruise collecting 196 samples during 31 dives.

Table 1. Dive summary for HBOI/DBMR from March 1984 to July 1989

	Scuba			Submersible (JSL)			ROV (Hysub)		
	# Dives	B.T. (hr)	# of Samples	# Dives	B.T. (hr)	# of Samples	# Dives	B.T. (hr)	# of Samples
1984	189	96.4	1158	38	88.6	968			
1985	384	220.4	1709	63	172.1	1426			
1986	235	139.7	2041	41	121.8	844			
1987	183	99.8	646	41	109.5	660	31	91.0	196
1988	350	211.6	1298	26	85.0	366			
1989	250	188.0	1103	34	97.6	349			
Total	1591	955.9	7955	243	674.6	4613	31	91.0	196
# Samples per dive	5.0			19.0			6.3		
# Samples per hr B.T.		8.3			6.8			2.1	

1) Scuba: # dives = total # of individual dives; B.T. = bottom time in hours of all divers.
2) Sub & ROV: B.T. = total dive times from launch to recovery.

A comparison of sampling efficiencies among the collection methods indicates that the submersible and scuba are similar in number of samples collected/hour of bottom time (6.8 and 8.3, respectively) (Table 1). The JSLs averaged 19 samples/dive compared to 5 samples/scuba diver. However, considering that the JSLs carry 4 individuals then the samples/diver are similar for scuba and submersibles. Although the ROV only collected 2.1 samples/hour of bottom time, it is expected that this could increase with experience and greater system reliability. This was a pilot cruise for HBOI's ROV and represented the first major collection expedition for scientific purposes ever made using an ROV.

Bioactivity Summary for 1988

During 1988, 2142 samples were collected by DBMR scientists at 147 sites off Colombia, Venezuela, Bahamas, Georgia, South Carolina, and Florida. Dominant taxa consisted of porifera (52.7%), cnidaria (19.2%) and algae (17.6%) (Fig. 1). Overall, 28-29% of all samples collected showed some activity in the primary screens within the antiviral (AV), antifungal (AF), antibacterial (AB), and immunomodulatory (IM) programs, and 17% activity for the P388 assay of the antitumor (AT) program (Table 2). Individual

assays within each program ranged from a low of 0.1% (3 samples) active for the ADNO assay to 27.4% (586 samples) for the BS assay.

Figure 1. HBOI/DBMR collections for 1988: Phylogenetic distribution

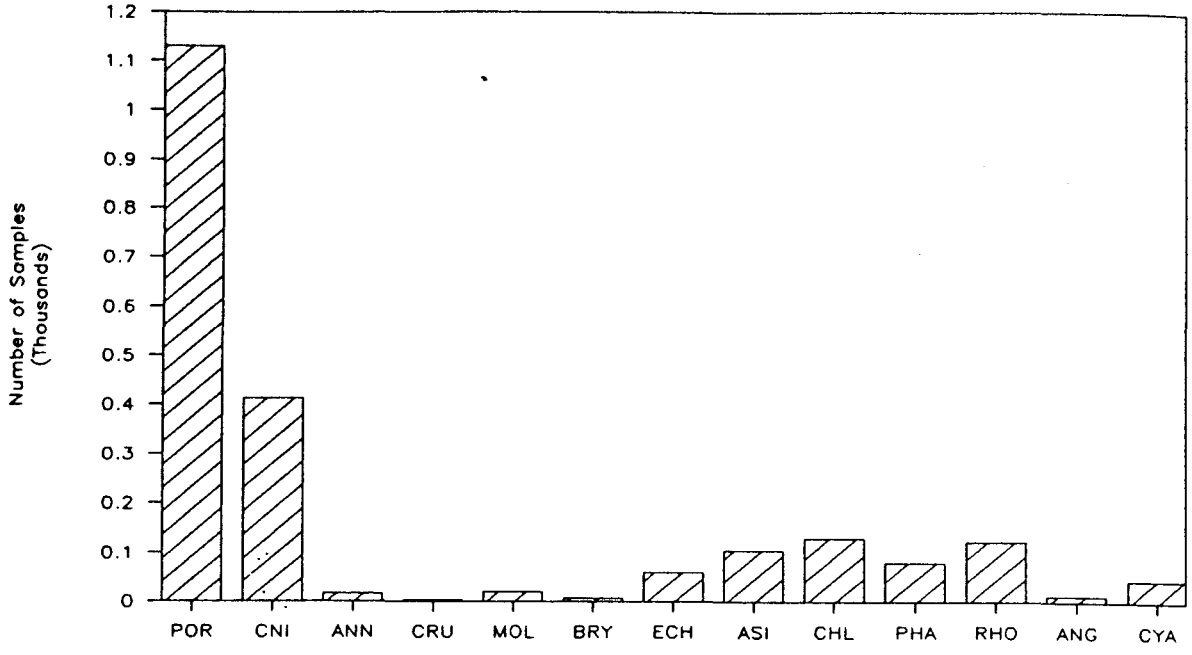

Figure 2. HBOI/DBMR collections for 1988: Bioactivity of phylogenetic distribution

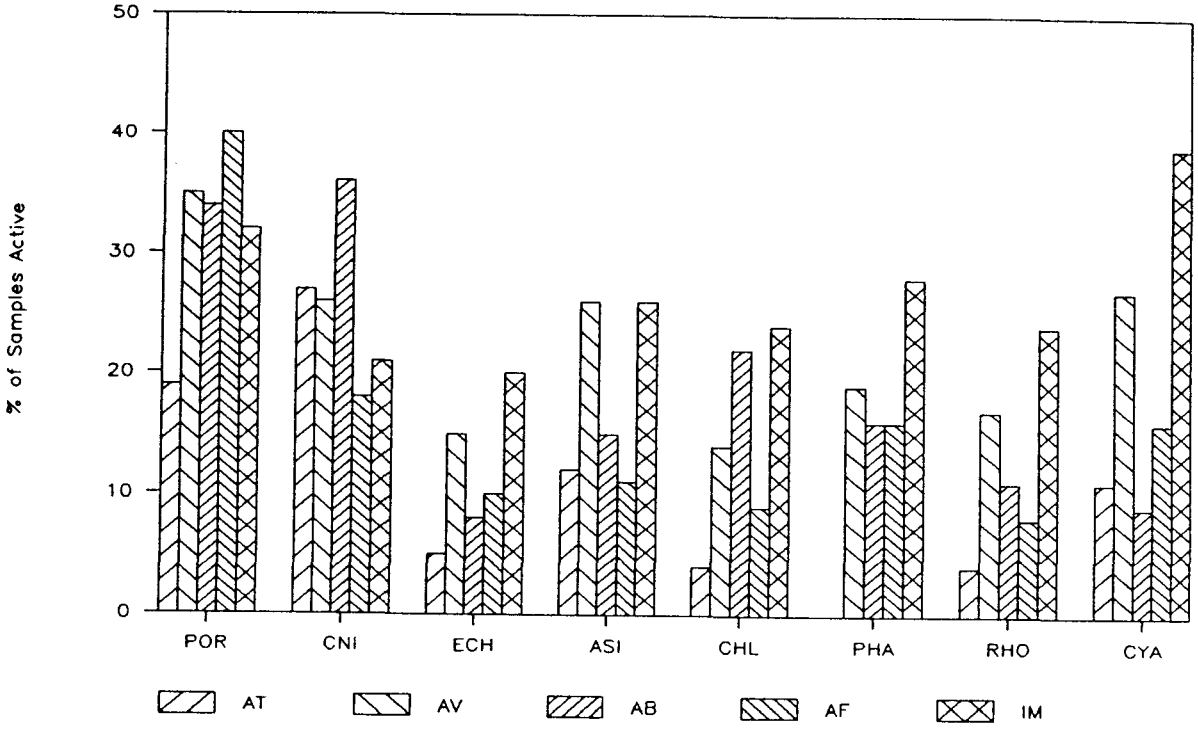

Table 2. Bioactivity summary for 1988 HBOI/DBMR collections by assay, depth and phylum

		DEPTH		PHYLUM												
		>100'	<100'	POR	CNI	ANN	CRU	MOL	BRY	ECH	ASI	CHL	PHA	RHO	ANG	CYA
TOTAL # SAMPLES		2142	735	1407	1130	412	16	3	20	6	60	105	130	80	124	12	44
		% ACTIVE															
PROGRAM	ASSAY																
Antitumor	P388	17	18	16	19	27	0	0	0	0	0	12	4	0	4	0	11
Antiviral	HSV	7	7	6	8	6	0	33	0	0	2	6	3	1	3	0	9
	A59	11	9	11	13	11	0	0	0	17	2	11	4	5	5	17	7
	FELV	12	15	11	15	9	13	33	15	0	10	11	5	10	8	33	14
	PR8	6	10	4	8	6	0	33	0	0	2	2	2	6	1	8	0
	ADNO	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
	TOTAL	29	34	26	35	26	13	67	15	17	15	26	14	19	17	42	27
Antibacterial	EC	6	7	6	10	2	0	0	0	0	2	4	0	0	2	0	0
	BS	27	31	26	32	35	0	0	10	0	7	15	22	15	11	17	9
	PA	5	8	3	8	2	0	0	0	0	2	1	0	1	0	0	0
	TOTAL	29	34	27	34	36	0	0	10	0	8	15	22	16	11	17	9
Antifungal	AN	10	13	8	13	8	6	0	15	0	8	8	1	0	2	0	5
	CA	12	17	10	16	12	0	0	0	0	5	3	3	1	6	17	11
	CA-RPM	26	34	21	38	16	0	0	0	0	10	9	6	16	5	50	14
	TOTAL	28	37	23	40	18	0	0	10	0	10	11	9	16	8	50	16
Immunomodulatory	ID	21	25	19	21	18	50	67	35	50	18	19	18	26	20	8	27
	IS	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0
	ID-IS	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0
	IS/NS	5	5	4	6	2	0	0	0	0	2	4	4	1	1	8	9
	TOTAL	29	34	26	32	21	50	67	45	50	20	26	24	28	24	17	39

POR - Porifera, CNI - Cnidaria, ANN - Annelida, CRU - Crustacea, MOL - Mollusca, BRY - Bryozoa, ECH - Echinodermata, ASI - Ascidiacea, CHL - Chlorophyta, PHA - Phaeophyta, RHO - Rhodophyta, ANG - Angiospermata, CYA - Cyanophyta

Table 3. Biologically active novel compounds discovered by HBOI/DBMR and affiliates

COMPOUND	SOURCE	BIOACTIVITY	REFERENCES
1. Cyclodercitin	Dercitus sp. (sponge) Bahamas,152m,JSL sub	P388	Gunawardana et al., (in press)
2. Nordercitin Dercitamin, Dercitamide	Stellata sp. (sponge) Bahamas,70m,JSL sub	P388,immunosuppressive	Gunawardana et al., (in press)
3. Bipinnatin A,B,C,D	Pseudopterogorgia bipinnata (gorgonian) Bahamas	P388	Wright et al., (in press)
4. Mycalamide A,B	Mycale sp. (sponge) New Zealand	P388 (in vivo), human tumor(in vivo-colon,mammary,lung)	Burres and Clement 1989
5. Onnamide	Theonella sp. (sponge) Japan	P388,human tumor cells(leukemia,lung, colon)	Burres and Clement 1989
6. Sulfircin	Ircinia sp. (sponge) Bahamas,119m,JSL sub	antifungal (Ca)	Wright et al., 1989
7. Brianthein V	Briareum asbestinum (gorgonian) Bahamas,2m	P388,A59	Coval et al., 1988
8. Topsentin, Bromotopsentin	Spongosorites sp. (sponge),Bahamas 174-355m,JSL sub	P388(in vivo), human tumor (in vivo-melanoma),HSV-1,VSV,A59	Tsujii et al., 1988
9. Discorhabin D	Latrunculia brevis (sponge),New Zealand, Prianos sp.(sponge),Japan	P388 (in vivo)	Perry et al., 1988
10.Manzamine E,F	Xestospongia sp. (sponge), Japan	P388	Ichiba et al., 1988
11.Manzamine A,B,C	Haliclona sp. (sponge),Japan	P388	Sakai et al., 1986,1987
12.Dercitin	Dercitus sp. (sponge) Bahamas,160m,JSL sub	P388(in vivo), HSV-1,A59, immunosuppressive	Gunawardana et al., 1988
13.Dragmacidin	Dragmacidon sp. (sponge) Bahamas,146m,JSL sub	P388,human tumor cells(lung,mammary,colon)	Kohmoto et al., 1988
14.Misakinolide A	Theonella sp. (sponge),Japan	P388,human tumor cells,antifungal(Ca)	Sakai et al., 1986
15.Reiswigins A,B	Epipolasis reiswigi (sponge) Bahamas,330m,JSL sub	HSV-1,VSV,A59	Kashman et al., 1987
16.Isospongiadol	Spongia sp. (sponge) Bahamas,203m,JSL sub	P388,HSV-1	Kohmoto et al., 1987
17.Curcuphenol, Curcudiol	Didiscus flavus (sponge) Bahamas,Belize 3-139m,JSL sub	P388,human tumor cells(lung,mammary, colon),antifungal(Ca)	Wright et al., 1987
18.Puupehenone	Strongylophora hartmani (sponge) 225m,JSL sub	P388 (in vivo),human tumor cells(lung,mammary, colon),antifungal (Ca)	Kohmoto et al., 1987
19.Azulene	Acalycigorgia sp. (gorgonian),Japan	P388,immunostimulatory, antifungal(Ca)	Sakemi and Higa, 1987
20.Peroxides	Plakortis sp. (sponge),Japan	P388	Sakemi et al., 1987
21.Duryne	Cribrochalina dura (sponge) Bahamas,12m	P388,human tumor cells (lung,mammary,colon)	Wright et al., 1987
22.Tubastrine	Tubastrea aurea (coral),Japan	HSV-1,VSV	Sakai and Higa, 1987
23.Venustatriol	Laurencia venusta (red algae),Japan	HSV-1,VSV	Sakemi et al., 1986

*Assays in vitro except where noted in vivo.
P388- mouse leukemia assay, HSV1- Herpes simplex type 1 virus,
A59- mouse corona hepatitus virus, VSV- vesicular stomatitus virus,
Ca- Candida albicans antifungal assay.

The distribution of bioactive samples for deep- and shallow-water collections were similar, although a slightly greater percentage of deep samples were active in nearly all bioassays (Table 2, Fig. 2). In general, the most bioactive phyla for all primary screens were porifera, cnidaria, ascidiacea, and algae (including blue-green algae). Other taxa such as annelida, crustacea, mollusca and bryozoa, which were relatively uncommon in the collections, were primarily active in the IM and AV programs. The most sensitive individual assays in terms of percentage of samples active for the various phyla were the FELV, BS, and CA-RPMI assays whereas the ADNO assay and immunostimulants (within the IM assay) rarely tested positive.

Culture Programs

DBMR's culture program has isolated and cultured over 170 strains of cyanobacteria and diatoms from macroorganisms and environmental samples. Seven separate strains including *Synechecoccus* sp. and *Nostoc* sp. have been scaled up to 250-l cultures. Strains of over 1000 heterotrophic microorganisms (fungi, bacteria, and actinomycetes) also have been isolated, cultured, and tested for bioactivity.

Within the sponge cell culture program starting last year, viable cells from 25 species of deep- and shallow-water sponges have been maintained in culture.

Novel Bioactive Compounds

By the end of 1988, DBMR and affiliates had determined 200 chemical structures of biologically active compounds from their collections. Nearly 50% of these were novel compounds. These have resulted in 14 issued and allowed patents and 44 publications on novel structures. Table 3 lists some of the novel compounds with bioactivity that were discovered by DBMR scientists.

DISCUSSION

Collections

HBOI's program for biomedical marine research has in a short span of 5 years discovered numerous bioactive novel compounds with potential for drug development (Table 3). Expeditions with the JOHNSON-SEA-LINK submersibles have resulted in the first major collections of deep-water organisms for marine natural product research (Boyd *et al.*, 1988). The sampling and photographic equipment outfitted on HBOI's ROV was developed for our program. This was the first ROV ever to be developed with such an array of tools specifically for scientific research.

Surprisingly, the sampling efficiencies for submersible and scuba were quite similar (Table 1). A true comparison of efficiencies among the different collection methods is difficult. Consideration also must be given to the quality of samples as well as to *in situ* documentation, cost and safety. Collections by the JSL submersibles are costly (Haskell *et al.*, 1987) but allow for complete documentation of deep-water samples with photographs, videotapes and environmental data. In the near future ROVs may approach JSL's capabilities at a cheaper price. ROVs, while limited by currents and lack of panoramic vision compared to the JSLs are capable of longer submerged time per operating day. Theoretically, with sufficient personnel, a research ROV could operate 24 hours a day. Limitations of scuba are depth, bottom time and safety. Dredge and trawl collections provide the least amount of *in situ* documentation and lower sample quality.

Table 4. Percentage of species active against antiviral (HSV-1), cytotoxic (P388), and antimicrobial (AM) assays from HBOI, New Zealand and Alpha Helix collections.

	HSV-1			P-388		AM		
	HBOI	NZ	AH	HBOI	NZ	HBOI	NZ	AH
Porifera	8	37	14	19	52	34	28	29
Cnidaria	6	3	17	27	21	36	6	11
Echinoderm	2	63	16	5	33	8	38	10
Ascidea	6	28	23	12	64	15	41	4
Bryozoa	0	27	0	0	39	0	19	0
Mollusca	0	18	0	0	42	10	8	3
Chlorophyta	3	25	7	4	17	22	17	7
Phaeophyta	1	52	25	0	64	16	37	3
Rhodophyta	3	22	17	4	45	11	27	7

1) HBOI- this study, 1988 data.
2) NZ- New Zealand, Munro et al. (1989); AM, using E. coli, P. aeroginosa, B. subtilis, or C. albicans.
3) AH- ALPHA HELIX Caribbean expedition, Rinehart (1988); AM, using E. coli, B. subtilis, Saccharomyces cerevisiae, Penicillium artrovenetum.

In addition to the objectives of the program, *i.e.*, drug discovery, several "spinoffs" resulted from the collections. Information on systematics, taxonomy, species distributions, ecology, behavior and geology have been compiled from over 1800 submersible and scuba dives. Museum vouchers deposited at the Smithsonian Institution, Washington, D.C. and HBOI's IRCZ museum represent the first extensive collection of deep-water marine organisms from the Galapagos Islands (Pomponi and van Hoek, 1987). The sponge voucher collection has resulted in the systematic revision of the Halichondrida using chemotaxonomic techniques (Pomponi et al., in press).

Bioassays

On-board bioassays are important to prevent the loss of ephemeral active compounds. On the R/V ALPHA HELIX expedition, Rinehart (1988) discovered that the bioactive compounds of a tunicate *Eudistoma* would have been missed without on-board screening because of rapid compound decay. Also, active species may be recollected and inactive species may be avoided during an expedition with on-board screening.

In an analysis of JSL submersible collections from the western Caribbean in 1985 and the Galapagos Islands in 1986, Rinehart (1988) reported that bioactive species were generally evenly distributed by depth at least to 760m. Our results from 1988 data actually indicate a slightly greater percentage of activity for all assay programs at depths >30m (Table 2). Rinehart (1988) and Munro *et al.* (1989) also reported an increase in antitumor (AT) activity but reduced antimicrobial (AM), antiviral (AV), and antifungal (AF) activities in deeper water. A review of our data does not support these conclusions.

A comparison of our 1988 bioactivity data with the ALPHA HELIX Caribbean expedition (Rinehart, 1988) and New Zealand collections (Munro *et al.*, 1989) shows that porifera, echinodermata, cnidaria, ascidiacea and algae were the dominant bioactive taxa (Table 4). However, some distinct zoogeographical differences are apparent in the bioactivities of various phyla. For example, in New Zealand, mollusca, bryozoa, and ascidiacea exhibited a much higher relative incidence of bioactivity and cnidaria a lower incidence of activity compared to the HBOI and ALPHA HELIX expeditions. Although there are differences in the relative distribution of dominant taxa for the subtropical-tropical collections in the Atlantic and Caribbean versus the temperate and sub-antarctic collections in New Zealand, the differences in the relative percentages of the active phyla are quite evident (Table 4). Furthermore, in a study of California sponges, Thompson *et al.* (1988) reported much higher antimicrobial activities than either of the Atlantic-Caribbean collections or the New Zealand study (70% overall antimicrobial; 23% *E. coli*, 53% *B. subtilis*).

Sources of Marine Natural Products

One barrier to the development of natural products as a source of drugs is availability (Suffness and Thompson, 1988). Various avenues must be explored to determine the feasibility of supplying sufficient quantities of pure active compounds for clinical trials of a potential candidate drug. Recollections may be considered if the compound comes from one or more species that are sufficiently abundant and are not endangered in nature.

Fermentation or culture of bioactive microorganisms is another potential source (Armstrong and van Baalen, 1979; Moore *et al.*, 1988). In some cases the activity associated with a macroorganism may result from a microbial symbiont. The bacterial content of some sponges, for example, may account for 38% of the sponge tissue volume (Bergquist and Wells, 1983). The feasibility of sponge tissue culture also is being researched (S. Pomponi), and aquaculture may be appropriate for some macroorganisms.

Another source of active compounds is chemical synthesis of novel compounds or their analogues. This is especially promising for simple compounds and polypeptides. Chemical synthesis has been achieved for the bioactive marine compounds prostaglandin from the octocoral *Plexaura* and manaolide from the sponge *Luffariella* (Garst *et al.*, 1986). Genetic engineering or gene splicing to produce active compounds from marine organisms has not been accomplished but may be an option in the future.

Potential for Drug Candidates

To date, only one marine-derived compound, didemnin B from the tunicate *Tridemnum*, has proceeded as far as NCI's phase II clinical drug trials to determine effectiveness against 22 human cancers (Suffness and Thompson, 1988). New screens with greater specificity for the mechanism of action for human diseases may provide a greater success rate of developing drugs from marine natural products.

One method is the molecular target approach, which uses biochemical markers as targets for potential antitumor agents. For example, HBOI discovered the novel compound topsentin from a marine sponge which inhibits tumors (Tsujii *et al.*, 1988). Its mechanism of action is through the inhibition of DNA topoisomerase which is an enzyme that is required for DNA replication by unlinking chromosomes prior to cell division.

Natural product research in the marine environment is relatively young and unexplored. The novel compounds discovered by HBOI and other investigators are

potential candidates for tomorrow's pharmaceutical drugs and agricultural products. Only through the cooperative efforts among biomarine research institutions, governmental research groups, and industry can mankind fully benefit from the exploitation of marine natural products.

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